

Review of Report, "Testing the uniqueness of *Z. h. intermedius* relative to *Z. h. campestris*," by R. R. Ramey II, H-P. Liu, L. Carpenter & C. W. Epps

Review Submitted by Mary V. Ashley, Ph.D., Dept. of Biological Science, University of Illinois at Chicago (May 30, 2005)

(1) Analyze the techniques used

The techniques used were appropriate and identical to those used in the Ramey et al. (2004a) report, with the exception that MDIV analysis was added. This study provides additional samples of *Z. h. intermedius* but no additional samples of *Z. h. preblei* or *Z. h. campestris*, so is only of ancillary relevance to the issue of delisting *Z. h. preblei*, in my opinion. I had previously suggested that additional markers would be useful (such as microsatellites), which has not been done, therefore this report provides only incremental strengthening of the genetic arguments for synonymizing *Z. h. preblei* and *Z. h. campestris*.

I am only moderately familiar with the MDIV method applied to the data set, although I have read several papers that have employed the method. The presentation given here to the method and the results is so minimal it is difficult to evaluate the findings. However, whenever I've seen MDIV applied to similar data sets, both estimated divergence times and gene flow are presented, as the model is designed to jointly obtain nonequilibrium coalescent estimates of divergence time and migration rate between pairs of populations. Therefore it is not clear why estimates of divergence times are not presented. Also I don't know why all pairwise subspecies comparisons are not included (e.g. comparisons of *Z. h. campestris* vs. *Z. h. pallidus*). MDIV results are usually presented as a matrix with one diagonal showing divergence times and the other showing migration rates. Finally, as a model-based method it is based on many assumptions, so results should be viewed with caution. The conclusions of the authors would be strengthened by additional analyses that included other available approaches that also supported their conclusions (e.g. *migrate*, nested clade analysis).

(2) Based on the data presented in the report do you support the authors' conclusions regarding synonymizing *Z. h. preblei*, *Z. h. campestris* and *Z. h. intermedius*?

Z. h. campestris has mtDNA haplotypes that are all over the tree, and shares haplotypes with *Z. h. luteus*, *Z. h. pallidus* and *Z. h. intermedius* and *Z. h. preblei*. *Z. h. luteus* and *Z. h. pallidus* were not included in the morphometric analysis, it is difficult to conclude that *Z. h. campestris* should be synonymized with *Z. h. intermedius* rather than one of the other subspecies. A new synonymized subspecies would not meet the criteria of being monophyletic for mtDNA. Of course, it may be that all subspecies should be subsumed, but that case is not made in this report and would require additional morphometric data. Another point is that *Z. h. preblei* and *Z. h. intermedius* do not share any mtDNA haplotypes, although they are not reciprocally monophyletic.

(3) Based on the MDIV data presented in the report, do you view *Z. h. prebleii* and *Z. h. campestris* as a single connected population?

As mentioned above, the presentation of the MDIV analysis is incomplete. The failure to reported estimated divergence times for the analysis might simply be an oversight but should be presented and the findings included in the discussion. As presented, the estimated migration rate between *Z. h. prebleii* and *Z. h. campestris* is quite low, so could be used to support either side of the argument (connectivity, or only low connectivity).

(4) Are there possible interpretations of the data? How likely are these possibilities?

One possible alternative interpretation of the data presented to date is that *Z. h. campestris* is not a valid subspecies but the rest are.

One troubling issue, given the importance that is being given to the mtDNA data, is the fact that all conclusions rest on the sequences obtained for 7 specimens of *Z. h. campestris* (KU109972, KU110013, KU123597, KU109978, KU123592, KU109984 and KU109985). These are the 7 that share *Z. h. prebleii* haplotypes. These appear to be from a single collection. I would like to know more about these samples. Who collected them and when? Given that specimens were thrown out routinely (13 in all, Ramey et al. 2004) because they didn't 'fit' and were presumed misidentified, is there any chance that these 7 were also misidentified as *Z. h. campestris*, or that the collection location was wrong? (or that the range of *Z. h. prebleii* extends to Custer Co, SD?). Given that *Z. h. campestris* haplotypes are found all over the tree, perhaps the ID's of this species at KU should be confirmed. Because all conclusions based on mtDNA, including the phylogeny, lack of reciprocal monophyly, AMOVA, and MDIV rest on the accuracy of these samples, they are worth a closer look.

(5) What additional analysis, if any, is needed to verify the study's assertions and why?

As mentioned in my previous report, additional genetic data including microsatellites would be very helpful. Additional analytical approaches, as mentioned above, for the mtDNA data would also be helpful.

(6) Has this new information changed your conclusions regarding the synonymizing of *Z. h. prebleii* and *Z. h. campestris* as proposed in Ramey et al. 2004a?

The case is now somewhat stronger indeed. It is noteworthy that the *Z. h. prebleii* mtDNA clade is nested within the *Z. h. intermedius* clade, although no haplotypes are shared. The situation is still complicated by the *Z. h. campestris* mtDNA haplotype data which cannot easily be reconciled with the proposed taxonomic revision.